Ver:1905

ViraTrap™ Lentivirus Purification Maxiprep Kit

（BW-V1270）

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Kit Contents

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Centrifugal Filters*	2	4	10
50 mL Centrifugal Tubes	1	4	5
Buffer P	45 mL	90 mL	225 mL
Buffer S	22 mL	44 mL	110 mL
Buffer MS	13 mL	26 mL	65 mL
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**Centrifugal Filters (Cat#BW-CF01)can be purchased from BEIWO separately.*

Introduction

Traditionally the recombinant lentivirus is purified by ultracentrifugation to separate the virus particles from cellular proteins and media components. The ultracentrifugation procedure is time consuming and limited to the amount of cell lysate to be processed, in addition, the ultracentrifugation also concentrates cellular debris, membrane fragments, and unwanted proteins from culture medium.

ViraTrap™ Lentivirus Purification Maxiprep Kit is designed for fast and efficient purification of recombinant lentiviruses from lentiviral-transfected cell culture supernatant. Viral particles can be purified from cell culture of 5 to 6 T75 flasks per column. The viruses are first applied to a purification column and then further purified and concentrated through a concentration unit.

Each column can be regenerated for purifying the same lentivirus. For optimized viral binding and recovery, each column can be regenerated only once.

Storage and Stability

The guaranteed shelf life is 12 months from the date of production. Store LV Maxi Columns, Buffer S, Buffer MS at 4°C, and all other components at room temperature (15-25°C).

Before Starting

Familiar with each step by reading this user manual and prepare all materials for the procedure.

Safety Information

The lentivirus infected cell media and the purified virus can be potential bio-hazardous material and can be infectious to human and animals. All protocols MUST be performed under at least Bio-Safety level 2 working condition.

Materials not Supplied

1. Standard TC centrifuge.
2. Swing bucket rotor.
3. 0.45 μm and 0.22 μm filter unit.
4. Rack holder for columns.
5. PBS.

Protocol

I. Harvest LV infected cell culture (for 5-6 T75 flasks per column)

1. Centrifuge the LV culture media at 3,000 rpm for 10 min at 4℃ and filter through a 0.45 μm filter unit. Transfer the supernatant into a clean tube and add **1 volume** of **Buffer P** to **3 volume** of **supernatant** (for example, add 10 mL Buffer P to 30 mL supernatant). Mix well and incubate at 4℃ for at least 4 h to overnight. The virus is stable in **Buffer P** for up to 1 week.

2. Centrifuge the samples at 4℃, 6,000 rpm for 30 min (Proceed to step **3** during centrifugation). Carefully aspirate the supernatant. Spin briefly and remove the residual supernatant carefully. The virus containing pellet should be visible and may appear hazy. Keep the virus on ice and proceed to step **4**.

II. Column preparation

3. Invert a **LV Maxi Column** to resuspend the resin inside the column. Put the column into a **50 mL Centrifugal Tube** and centrifuge at 4℃, 500 × g for 2 min. Tear off the tip on the bottom of the column and place the column into the **50 mL Centrifugal Tube**. Loosen the cap to allow buffer drain out from the column by gravity. Once the liquid stops dripping, add **6 mL Buffer S** evenly to the column and let it drain out by gravity without drying the column out.

   Note: A **Press-On Cap** for the bottom tip of the column is provided for stopping the gravity flow at any time.

4. Resuspend pellet from step **2** with **4 mL Buffer S** completely. Spin the sample at 4℃, 3,000 rpm for 5 min and transfer the supernatant to a clean vial. Keep the virus on ice.

5. Apply the sample to a **Centrifugal Filter** and spin at 4℃, 3,000 rpm for 15-20 min till 500 μL sample remains in the reservoir.

III. Load the sample to the purification column

6. Transfer the sample evenly to the **LV Maxi Column** and let it flow into resin by gravity. Once the entire sample gets into the resin, proceed to next step.

   Note: Slowly add the sample dropwise to the resin. Once the entire sample gets into the matrix, proceed to next step. Do not let the column dry out.

IV. Elute LV from the purification column

7. Add **6 mL Buffer MS** evenly to the **LV Maxi Column** and collect 6 mL flow-through. The virus is in the flow through liquid. Keep the virus on ice.

V. Concentration

8. Apply 4 mL sample collected from step **7** to the reservoir of a **Centrifugal Filter** and centrifuge at 4℃, 3,000 rpm for 10 min, process the remaining sample as described. Continue to spin the sample at 4℃, 3,000 rpm for 10–15 min till approximately 500 μL remains in the

reservoir. Pipet the solution up and down several times in reservoir and transfer the virus containing solution to a clean vial. The purified virus is ready for downstream applications.

Note: Always centrifuge less time and check the liquid level, do not let the solution dry out. Continue to centrifuge till the desired volume is achieved.

Note: If not using the Centrifugal Filter, the virus can also be desalted by dialysis or other desalting columns.

Note: A swing bucket rotor is preferred. Fixed angle rotor requires higher speed of 7,000 rpm for 15-20 min.

Note: Time for centrifugation may vary for different type of rotors.

- Typical Concentration Volume Vs. Spin Time (Swing bucket rotor, 4℃, 3,000 rpm， 4 mL starting volume) for 100K Centrifugal Filter device

  Spin time-15 min: concentrate volume 176 µL

  Spin time-20 min: concentrate volume 76 µL

  Spin time-25 min: concentrate volume 58 µL

- Typical Concentration Volume Vs. Spin Time (35° Fixed angle rotor, 4℃, 7,000 rpm, 4mL starting volume) for 100K Centrifugal Filter device

  Spin time-10 min: concentrate volume 97 µL

  Spin time-15 min: concentrate volume 54 µL

  Spin time-20 min: concentrate volume 35 µL

9. Aliquot and store the final purified virus at -80°C. Before infect target cells, we recommend adding the needed amount of purified virus to 5-10 mL culture medium of your target cells and filter through a 0.22 µm sterile filter before infection.

VI. Regeneration of the column

10. Upon completion of the purification procedure, add **5 mL Regeneration Buffer** to the column and let the buffer passes through the column by gravity flow. Wash the column by 10 mL PBS, and let the PBS pass through the column by gravity flow. Once the liquid stops dripping, fill the column with 3-5 mL PBS. Press on the cap to the bottom. Screw on the cap and wrap the column with parafilm in a zip block bag and store at 4℃.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.

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